## FOLIAGE, POD AND INTERNAL SEED INFECTION OF SELECTED COMMON BEAN LINES WHEN INOCULATED WITH TWO STRAINS OF XANTHOMONAS AXONOPODIS PV. PHASEOLI

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Common bacterial blight (CBB) is an important foliar and seed-borne disease of common bean (Phaseolus vulgaris L.) grown in tropical, subtropical and temperate areas. The disease is caused by Xanthomonas axonopodis pv. phaseoli. Most common bean cultivars are currently susceptible to the bacterium. The bean breeding program at the University of Puerto Rico has developed CBB resistant breeding lines using VAX 6, VAX 3 and WBB-20-1 as sources of resistance. The objective of this study was to determine the level of resistance incorporated into these lines using strains of Xap prevalent in Puerto Rico and to identify lines with the most resistance in foliage, pods and seeds. Two strains from Puerto Rico of the common type of Xap (484 and 3353) were selected for inoculation at 1.0 A and 590 nm. Leaves were inoculated with 10<sup>7</sup> CFU/ml using multiple needles and scored at 9 days using the CIAT scale (1-9). Green pods (6-8 cm) attached to plants were inoculated before the expression of seed formation (flat) using pipette tips to place 5µl of inoculum on three sites on the pod surface without penetration. Lesion diameters were evaluated at 7 and 14 days using a 1-9 scale where 1 = 0 mm, 2 = 1.5 mm, 3 = 2 mm, 4 = 2.5 mm, 5 = 3 mm, 6 = 3.5 mm, 7= 4 mm, 8 = 4.5mm, and  $9 \ge 5$ mm. A selected number of pods were harvested at maturity and maintained at 5°C until used to determine the percentage of internal bacterial seed infection. Eight susceptible lines for each bacterial strain and their respective non inoculated controls were evaluated as well as eight resistant genotypes and their respective non-inoculated genotypes using individual pods and seeds. The cultivar 'Morales' was included as a susceptible check. For bacterial internal detection 100 µl of 10<sup>-1</sup> dilutions of seeds previously disinfested were placed on yeast dextrose calcium carbonate agar.

No significant difference in virulence was detected between the strains when inoculated on leaves and pods (Table 1). However, line PR 0443-38 showed resistance to Xap 484 and susceptibility to Xap 3353 on pods which suggests a differential reaction on the pods to the strains. A significant difference in the ability of the strains to infect internal seed tissues was observed. Internal seed infection was detected only when pods were inoculated with Xap 484. The level of seed infection on susceptible lines ranged from 16.6 to 100.0% (Table 1). One hundred percent of the Morales seed was infected when inoculated with Xap 484, whereas no seed of Morales was infected with Xap 3353. None of the bean lines with susceptible reactions to Xap 3353 on the pod surface had internal seed infection. All of the breeding lines with resistant pods were also resistant to internal seed infection for both strains. Xap 3353 was less compatible on the seed tissue than in the parenchyma pod tissues. This observation is supported by the reactions of other susceptible breeding lines that were also resistant to internal seed infection by Xap 3353 strain. The most important difference between the two strains of Xap is their ability to colonize internal seed tissue. In practice, it is very difficult to distinguish between the strains and both were previously determined to belong to the same rDNA ribogroup using EcoR1. Survival of the strains under natural conditions may differ. Primary inoculum for Xap 484 may depend on seed infection whereas Xap 3353 may depend mainly on seed surface contamination and contact with infected residues for primary inoculum. The resistant reaction on leaves, pods and internal seed infection has a very important implication in the reduction

of bacterial inoculum under field conditions. The most promising lines with resistant reactions on both leaves, pods and no internal seed infection were PR0443-17, derived from the cross 'VAX 5 /// DOR 483 / BelNeb RR2 // MUS 83 / DOR 483'; PR0443-73, derived from the cross 'VAX 6 /// DOR 483 / BelNeb RR2// MUS 83 / DOR 483; and PR0443-3, derived from the cross 'DOR 364 / WBB-20-1 // DOR 482 /// VAX 6'. These white-seeded bean breeding lines have the SR-2 SCAR marker for the *bgm* gene and the SW12 QTL for Bean Golden Yellow Mosaic Virus resistance, the SW13 SCAR marker for the *I* gene resistance to Bean Common Mosaic Virus and the SAP 6 QTL for CBB resistance. Only PR0443-17 has the SU-91 SCAR marker for CBB resistance. These breeding lines produce seed yields equal to or better than Morales, the preferred white bean cultivar in Puerto Rico.

**Table 1.** Mean leaf and pod reactions and percentage of seed infection of common bean lines inoculated with two strains of the common type of *Xanthomonas axonopodis* pv. *phaseoli* 

Interaction	Pod reaction <sup>1</sup>		% seed	Leaf reaction <sup>3</sup>	
	7DAI	14 DAI	infection <sup>2</sup>		
Xap 484 Group 1					
Compatible lines	<b>Xap 484</b>		Xap 484	Xap 484	Xap 3353
Morales	3.00	4.05	100.0	7.47	$\bar{7}.90$
PR0443-110	2.75	4.05	16.6	5.00	4.65
PR0443-7	2.51	2.70	60.0	3.17	2.90
PR0313-95	3.50	4.20	50.0	7.47	7.20
Incompatible lines					
PR0443-17	1.0	1.0	0.0	1.07	1.20
PR0443-7	1.0	1.0	0.0	3.17	2.90
PR0443-38	1.0	1.0	0.0	2.25	2.33
PR0443-73	1.0	1.0	0.0	1.76	1.30
Xap 3353 Group2					
Compatible lines	Xap 3353		Xap 3353	Xap 484	Xap 3353
Morales	3.26	4.80	0.0	7.47	$\bar{7}.90$
PR0443-38	2.25	3.00	0.0	2.25	2.33
PR0443-110	2.25	2.55	0.0	5.00	4.65
PR0309-4	2.25	4.50	0.0	3.20	1.90
Incompatible lines					
PR0443-3	1.00	1.00	0.0	1.20	1.10
PR0443-105	1.00	1.00	0.0	3.40	3.67
PR0443-103	1.00	1.00	0.0	4.30	3.20
W-BB-20-1	1.00	1.00	0.0	3.30	3.80

Pod reaction refers to mean severity scores observed on pod surfaces at 7 and 14 days after inoculation (DAI) under greenhouse conditions where 1 = no symptoms and  $9 = \text{lesion size} \ge 5 \text{ mm}$ .

## References

Centro Internacional de Agricultura Tropical (CIAT). 1987. Standard system for the evaluation of bean germplasm. Van Schoonhoven and M.A. Pastor-Corrales (compliers). Cali. Colombia 54p.

Saettler, A.W., Schaad, N.W., and Roth, D.A. 1989. Detection of bacteria in seed and other planting material. APS Press. St. Paul, Minnesota.

<sup>&</sup>lt;sup>2</sup> Seed % infection refers to the percentage of internal seed infection detected under *in vitro* conditions.

 $<sup>^{3}</sup>$ Mean leaf reactions to two Xap strains at nine days after multiple needle inoculation. The CIAT 1-9 scale was used where 1 = no symptoms and 9 = severe symptoms on the leaves.